

Research Article

Antioxidant and Antimicrobial Properties of Cactus Pear (*Opuntia*) Seed Oils

Esther Ramírez-Moreno,¹ Raquel Cariño-Cortés,² Nelly del Socorro Cruz-Cansino,¹ Luis Delgado-Olivares,¹ José Alberto Ariza-Ortega,¹ Vanessa Yelina Montañez-Izquierdo,³ María Manuela Hernández-Herrero,³ and Tomás Filardo-Kerstupp⁴

¹Academic Area of Nutrition, Health Sciences Institute, Autonomous University of Hidalgo State, 42160 Pachuca, HGO, Mexico

²Academic Area of Medicine, Autonomous University of Hidalgo State, Eliseo Ramírez Ulloa 400, 42090 Pachuca, HGO, Mexico

³Department of Food Hygiene, Faculty of Veterinary, Autonomous University of Barcelona, 08193 Bellaterra, Spain

⁴Academic Area of Chemistry, Basic Science and Engineering Institute, Autonomous University of Hidalgo State, Carretera Pachuca-Tulancingo Km. 4.5, Mineral de la Reforma, 42183 Pachuca, HGO, Mexico

Correspondence should be addressed to Nelly del Socorro Cruz-Cansino; ncruz@uaeh.edu.mx

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Seed oils from two Mexican varieties of cactus pear (green: *Opuntia albicarpa* and red: *Opuntia ficus indica*) were extracted with different solvents (hexane, ethanol, and ethyl acetate) to evaluate their antioxidant activity. The seed oil with higher antioxidant activity was selected to evaluate antimicrobial activity. The fatty acid profile was analyzed by gas chromatography-mass spectrometry (GC-MS). Oil from green cactus pear seeds obtained with ethanol and ethyl acetate exhibited higher antioxidant activity ($p < 0.05$) of 323 and 316 $\mu\text{mol TE}/20 \text{ mg}$ ($p < 0.05$), respectively, compared to red cactus pear seed oil (≈ 274 and 247 $\mu\text{mol TE}/20 \text{ mg}$ with ethyl acetate and ethanol, resp.). The oil obtained with ethanol and higher antioxidant activity was used to determine the antimicrobial activity. Both cactus pear oils produced a microbial inhibition zone in most of the microorganisms evaluated, particularly *Saccharomyces cerevisiae* which had similar diameter (38–40 mm). The oil fatty acids profiles of both varieties were similar and exhibited a high content of linoleic acid, while two fatty acids (linolenic and behenic) found in red cactus pear were not observed in the green variety.

1. Introduction

A relatively untapped source of lipid and protein raw material is the by-product of fruit-processing plants. Millions of pounds of fruit seeds are discarded yearly causing disposal problems, while proper utilization of these waste products could lead to an important new source of oil and meal [1]. Seeds of fruits collect at least some cytoplasmic lipid bodies as major storage reserve for lipid accumulation [2]. Fruit seeds oils are of great interest because they are edible oils with high degree of unsaturation, antioxidant radical scavenging properties [3–8], and a broad spectrum of antimicrobial activity [9–15]. Therefore, the oil from plants can be potentially used by the food industry for the manufacturing of “natural” or “green” safe foods [16] and extend shelf-life [17, 18].

The oil from cactus pear seed has been found to have an appreciable amount of oil with high levels of unsaturated fatty acids [19], with antioxidant [20, 21] and antimicrobial activity [22], as well as cardioprotective, antithrombotic, anti-inflammatory, antiarrhythmic, hypolipidemic, and antihyperglycemic effect [23, 24]. These properties are of interest for the pharmaceutical and food industry. However, the concentration and effectiveness of these oils may vary among cultivars or varieties, crop environmental factors (e.g., light, temperature, and type of soil nutrients), or methods and solvents used for their extraction. Therefore, the purpose of this research was to determine the antioxidant and antimicrobial activity, and fatty acid profile of the oil obtained from two Mexican varieties of cactus pear (*Opuntia albicarpa* and *Opuntia ficus indica*) seeds extracted with different solvents.

2. Materials and Methods

2.1. Plant Material. Two Mexican varieties of cactus pear (*Opuntia albicarpa* and *Opuntia ficus indica*) fruit, green (*cv.* Reyna) and red (*cv.* Rojo Pelón), respectively, were provided by the Mexican association CoMeNTuna (Consejo Mexicano del Nopal y la Tuna, A.C.; Actopan, Hidalgo, Mexico). Fruits free of external injuries were selected, washed, and manually peeled. Cactus pear seeds were obtained after juice was extracted stirring the pulp with an industrial blender (38BL52 LBC10, Waring Comercial®, USA) and passing it through a conventional strainer. The seeds retained were washed in the strainer with water until pulp residues were removed.

2.2. Powder Seed and Oil Extraction. Green cactus pear seeds (GCPS) and red cactus pear seeds (RCPS) were sun-dried and then grounded (Cyclotec 1093, Tecator Sweden) to a 1 mm diameter mesh and stored at -32°C until further analysis. The seed oil was extracted as follows: 25 g of powdered seeds was mixed with 500 mL of solvents with varying polarities (hexane, ethanol, and ethyl acetate) and the obtained residue was reextracted until extraction solvents become colourless. All the extracts were filtered through filtration paper Whatman number 1 and the filtered extracts were collected for further drying and removal of the remaining solvent at 50°C using a rotary evaporator (BUCHI, R-200, Switzerland). All extracts were placed in plastic bottles and then stored at -20°C until used. The oils obtained were used to further analysis.

2.3. Free Radical Scavenging Assay. The free radical scavenging activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH^{*}) radical as described by Morales and Jiménez-Pérez [25]. A volume of 500 μL of ethanolic DPPH^{*} solution (7.4 mg/100 mL) was added to a sample aliquot of 100 μL placed in vials. The mixture was left to sit at room temperature for 1 h and then was vortexed and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 520 nm in a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA), and μmol of Trolox equivalents per 20 milligram ($\mu\text{mol TE}/20\text{ mg}$) of sample was obtained. Oil samples with best antioxidant capacity obtained from the different solvents were used for the antimicrobial analysis.

2.4. Antimicrobial Activity. Eight standard freeze-dried cultures of bacteria, *Candida albicans* (ATCC 10231), *Escherichia coli* O58:H21 (ATCC 10536), *Escherichia coli* O157:H7 (CCUG 44857), *Staphylococcus aureus* (ATCC 13565), *Listeria monocytogenes* (CCUG 15526), *Pseudomonas aeruginosa* (ATCC 15442), *Saccharomyces cerevisiae* (CECT 1942), and *Salmonella Typhi* (CCUG 29478) were obtained in thermostealed vials from the Spanish Type Culture Collection (Autonomous University of Barcelona, Barcelona, Spain). Freeze-dried cultures were rehydrated in tryptone soy broth at 37°C for 18 h and then were used to inoculate tryptone soy agar and malt extract agar plates; all microorganisms were incubated at 37°C except *Candida albicans* and *Saccharomyces cerevisiae* which were incubated at 25°C . Individual colonies

were maintained on specific agar slants, stored at 4°C , and subcultured every 15 days.

Disc Diffusion Assay. Antimicrobial activity of oil extracted from GCPS and RCPS was carried out using the disc diffusion method [26]. Petri plates were filled with $\sim 20\text{ mL}$ of sterile tryptone soy agar for bacteria and malt extract agar for fungi. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 minutes. Serial dilutions (10–50 $\mu\text{g}/\text{mL}$) of the seed oil from a stock solution (1 mg/mL) were prepared in 20% DMSO and 10 μL loaded onto the sterile blank discs (BBL™ Sensi-Disc™) of 6 millimeters of diameter. On the media surface the loaded disks were placed and left for 30 minutes at room temperature to allow compound diffusion. The seed oil was serially diluted in Mueller–Hinton broth medium and duplicate tubes of each dilution (10–100 $\mu\text{g}/\text{mL}$) were inoculated with 5×10^6 cells of the test bacteria strain and cultures. The antibiotic agents Sensi-Disc streptomycin, ampicillin, and sulfamethoxazole/trimethoprim (BBL Sensi-Disc) were used as positive controls at the same concentration level. After plates were incubated at 37°C for 24 h, the diameters of the inhibition zones were recorded in millimeters. Three independent repetitions were performed and tests were made in triplicate.

2.5. GC-MS Analysis. The GC-MS analysis was performed with a GC-MS HP-5890 (Hewlett-Packard Company, Palo Alto, California, USA) equipped with a Flame Ionization Detector (FID), and a ZB-WAX fused silica capillary column (60 m \times 0.25 mm i.d. \times 0.25 mm film thickness) packed with 5% phenylmethylpolysiloxane (Phenomenex, Torrance, CA). To obtain the methyl esters, the cactus pear seed oils were saponified and derivatized using KOH 1N (IUPAC, 1969). Changes in the fatty acids of the oils samples were compared against a standard mixture of 37 components of fatty acids methyl esters (FAMES) (Food Industry FAMES Mix, Restek) comprised by methyl esters with chains C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1n9c, C18:1n9t, C18:2n6c, C18:2n6t, C18:3n6, C18:3n3, C20:0, C20:1n9, C20:2, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C21:0, C22:0, C22:1n9, C22:2, C22:6n3, C23:0, C24:0, and C24:1n9. The sample volume injected was of 2 μL (split ratio 20 : 2) at an injector and detector temperatures of 225 and 225°C , respectively. N_2 was used as carrier gas at a flow rate of $1.2\text{ mL}\cdot\text{min}^{-1}$. Fatty acids were calculated as percentage of total FAMES.

2.6. Statistical Analysis. All values were obtained by triplicate and expressed as means \pm standard deviations (SD). Data were analyzed using the SPSS V.15 software (SPSS Institute Inc., Cary, NC). An ANOVA was carried out to determine differences between oils extracted as well as its antimicrobial activity that were significant at the 5% level of probability and a Tukey test was used for comparison of data.

3. Results and Discussion

3.1. Yield Comparison between Extraction Solvents. Hexane, ethanol, and ethyl acetate were used to extract the oil from

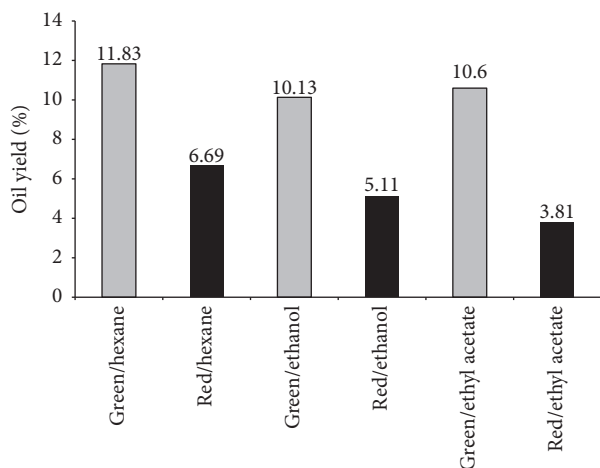


FIGURE 1: Oil yields (%) extracted from GCPS and RCPS with hexane, ethanol, and ethyl acetate.

cactus pear seeds. The extraction yields are compared in Figure 1, which shows that the higher amount of oil (%) was obtained from the green cultivar and that yield depended on the solvent used. Oil extraction with hexane was higher for both fruit varieties (11.83% for GCPS and 6.89% for RCPS), followed by ethanol, which reached the same yield as ethyl acetate for GCPS ($\approx 10\%$). Ethyl acetate was the least effective solvent for RCPS. The extraction yields were similar to those reported (≈ 7 to 11%) for several varieties of *Opuntia ficus indica* [27–29]. This extraction yields will vary depending on several factors as fruit variety, harvest period, maturation, geographic region, percentage of oil in the seed, and chemical compounds found in the source and by the extraction method [30]. Researchers have determined that solvent extraction combined with other methods could increase oil yield, as high pressure or supercritical fluid combined with solvent reached a yield of 9.33% from tobacco seeds (*Nicotiana tabacum* L.) while sonication and Soxhlet reached a 7.75% and 13.72%, respectively [31].

3.2. Free Radical Scavenging Activity. Solvent extraction is usually used for isolation of antioxidants; the extraction depends on the solvent selected based on the different antioxidant compounds with varying polarity [32, 33]. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [34, 35]. The DPPH assay has also been used to predict the oxidative stability of edible oils [36, 37]. The antioxidant activity determined by DPPH of the oil extracted from RCPS and GCPS is shown in Figure 2. Oil from the GCPS extracted with ethanol and ethyl acetate exhibited the higher antioxidant activity ($p < 0.05$) of 323 and 316 $\mu\text{mol TE}/20\text{ mg extract}$, respectively, followed by RCPS oil extracted with ethyl acetate (274 $\mu\text{mol TE}/20\text{ mg extract}$) and ethanol (247 $\mu\text{mol TE}/20\text{ mg extract}$). These results demonstrate that the extraction solvent had a significant effect on the free radical scavenging capacity of the oil, where the hexane had the lower values. In our study, the green variety exhibited a higher antioxidant activity regardless of solvent. Different results may depend mainly

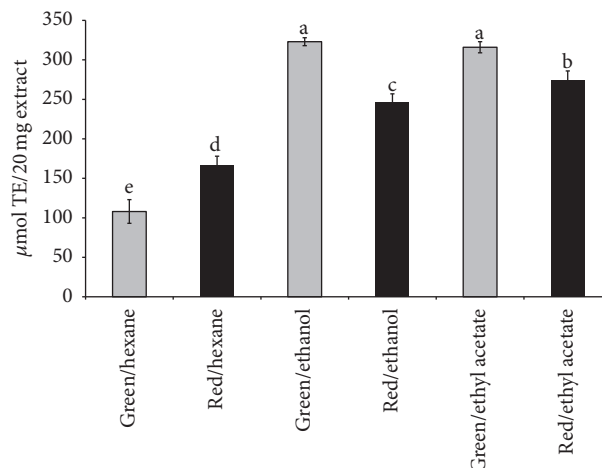


FIGURE 2: Antioxidant activity of GCPS and RCPS oils extracted with different solvents. ^{a-d}Different letters above bars indicate that samples are significantly different ($p < 0.05$).

on the content and concentration of bioactive compounds in the oil, but other factors such as solvent polarity, solubility of the extracts in different testing systems, stereoselectivity of the radicals [38], and strong synergism between fatty acids [6] may affect antioxidant activities. Other studies have also reported diverse antioxidant activity among oils from different *Opuntia varieties* [20, 39, 40].

3.3. Antibacterial and Antifungal Activity. The most recommended way to prevent or inhibit microbial growth in foods is the use of food preservatives. Essential oils are secondary metabolites of plants that have wide applications in the food flavoring and preservative industry [41]. Six different bacterium and two fungi species were used to screen the antimicrobial potential of the oils extracted from the two varieties of cactus pear seeds. Oil extracted with ethanol exhibited the highest antioxidant activity and therefore it was used to evaluate the antibacterial and antifungal activity. Figure 3 shows the results from the microbial assay where most microorganisms showed an inhibition zone when exposed to GCPS and RCPS oils, except *Salmonella Typhi* and *Escherichia coli* O157:H7 (image not shown). From these two microorganisms, the first showed an inhibition zone in the presence of antibiotic agents streptomycin (S), ampicillin (AMP), and sulfamethoxazole/trimethoprim (STX) in diameters of 14.6, 11.3 and 27.3 mm, respectively (Table 1), while *Escherichia coli* O157:H7 was only inhibited by SXT (25.3 mm), which agrees with other reports of multiantibiotic resistance of *E. coli* O157:H7 due to the presence of the gene cluster AMR-SSuT [42] and production of beta-lactamase [43]. On the other hand, *Saccharomyces cerevisiae* was highly inhibited (38–40 mm) by the extracted oils but grew in presence of the antimicrobial agents (Figure 3). Similar results were observed for *Candida albicans*, although inhibition zones were smaller and similar for both oils. These observations demonstrate that certain compounds in the cactus pear seed oil have antimicrobial activity. Other researchers also reported similar observations for cactus

TABLE 1: Diameters of growth inhibition zones (mm) in the presence of oil extracted from cactus pear seeds and conventional antimicrobials^A.

Microbial cultures	Extract			Antimicrobial agent	
	GCPS	RCPS	S	AMP	SXT
<i>Saccharomyces cerevisiae</i> (CECT1942)	38.3 ± 4.2 ^a	40.3 ± 4.5 ^a	ND	ND	ND
<i>Escherichia coli</i> O58:H21 (ATCC 10536)	11.9 ± 0.7 ^d	11.4 ± 0.9 ^d	19.5 ± 1.4 ^b	17.8 ± 1.5 ^c	29.9 ± 0.9 ^a
<i>Escherichia coli</i> O157:H7 (CCUG 44857)	ND ^B	ND	ND	ND	25.3 ± 1.0
<i>Staphylococcus aureus</i> (ATCC 13565)	12.1 ± 1.0 ^c	11.1 ± 1.1 ^c	18.3 ± 0.9 ^b	28.1 ± 1.5 ^a	33.3 ± 1.9 ^a
<i>Listeria monocytogenes</i> (CCUG15526)	13.3 ± 1.5 ^c	11.4 ± 0.9 ^d	21.3 ± 1.2 ^a	17.3 ± 0.8 ^c	37.6 ± 1.8 ^a
<i>Pseudomonas aeruginosa</i> (ATCC15442)	16.4 ± 2.1 ^c	15.1 ± 2.0 ^c	20.1 ± 1.5 ^b	24.1 ± 2.5 ^a	36.6 ± 2.9 ^a
<i>Salmonella typhi</i> (CCUG29478)	ND	ND	14.6 ± 0.4 ^b	11.3 ± 1.8 ^c	27.3 ± 0.8 ^a
<i>Candida albicans</i> (ATCC 10231)	11.1 ± 1.0 ^a	11.0 ± 1.8 ^a	ND	ND	ND

^AInhibition zone diameters for oil and reference antibiotics are means ± SE of three replicas. GCPS: green cactus pear seed oil extract, RCPS: red cactus pear seed oil extract, S: streptomycin (10 µg/disc), AMP: ampicillin (10 µg/disc), and SXT: sulfamethoxazole/trimethoprim (10 µg/disc). ^BND: not detected activity. ^{a-d}Different letters in the same row indicate significant differences.

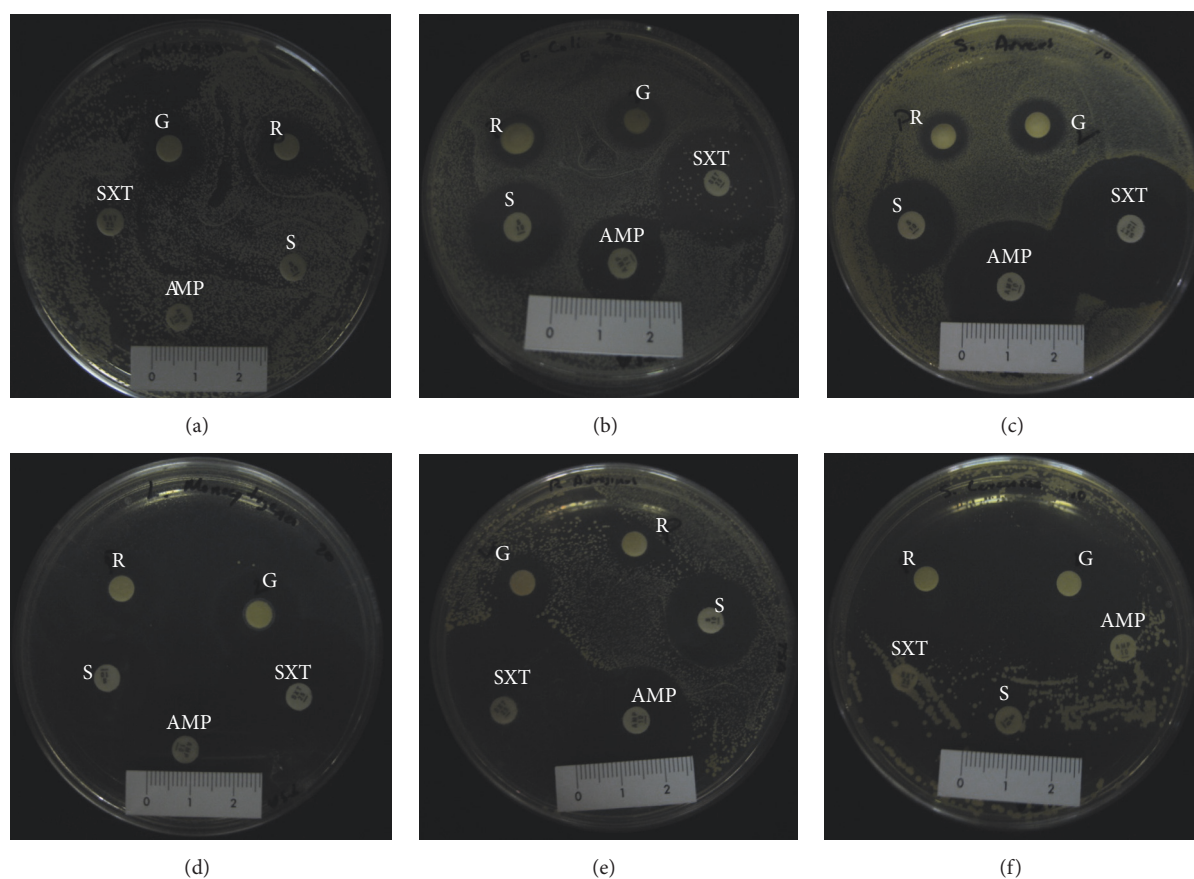


FIGURE 3: Antimicrobial activity of oil extracted from green cactus pear (G); oil extracted from red cactus pear seeds (R); streptomycin (S); ampicillin (AMP); and sulfamethoxazole/trimethoprim (SXT). *Candida albicans* (ATCC 10231) (a); *Escherichia coli* O58:H21 (ATCC 10536) (b); *Staphylococcus aureus* (ATCC 13565) (c); *Listeria monocytogenes* (CCUG15526) (d); *Pseudomonas aeruginosa* (ATCC15442) (e); *Saccharomyces cerevisiae* (CECT1942) (f).

pear fruit cv. *Opuntia stricta* [44] and for other plants as fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.) [5]. Differences in the levels of antimicrobial activity may be partially attributed to variable chemical composition of the oils [45]. Mnayer et al. [46] suggested that oil compounds can act on different bacterial structures, while Gill et al. [47] mentioned that whole oils have a greater

antibacterial activity than the major component mixed, so that minor components are critical for the activity and exert a synergistic effect [16, 48, 49].

In the present study, the antimicrobial activity of cactus pear seed oil was more effective against fungi compared to bacteria cultures. These interesting results suggest that there is a link between the oil chemical contents and the

TABLE 2: Percentages of FAMES in crude cactus pear seed oil extracts.

FAMES	Green cactus pear seed oil extract	Red cactus pear seed oil extract
C14:0	0.078 ± 0.00	0.066 ± 0.01
C16:0	12.327 ± 0.09	12.887 ± 0.02
C16:1	0.429 ± 0.02	0.570 ± 0.01
C16:2	0.073 ± 0.00	0.540 ± 0.00
C17:0	0.060 ± 0.01	0.075 ± 0.00
C18:0	3.436 ± 0.01	3.389 ± 0.07
C18:1	16.215 ± 0.03	17.061 ± 0.01
C18:2	67.448 ± 0.08	65.407 ± 0.01
C18:3	Ni	0.372 ± 0.01
C22:0	Ni	0.160 ± 0.01

Means of 3 replicates ± SE. Ni: not identified.

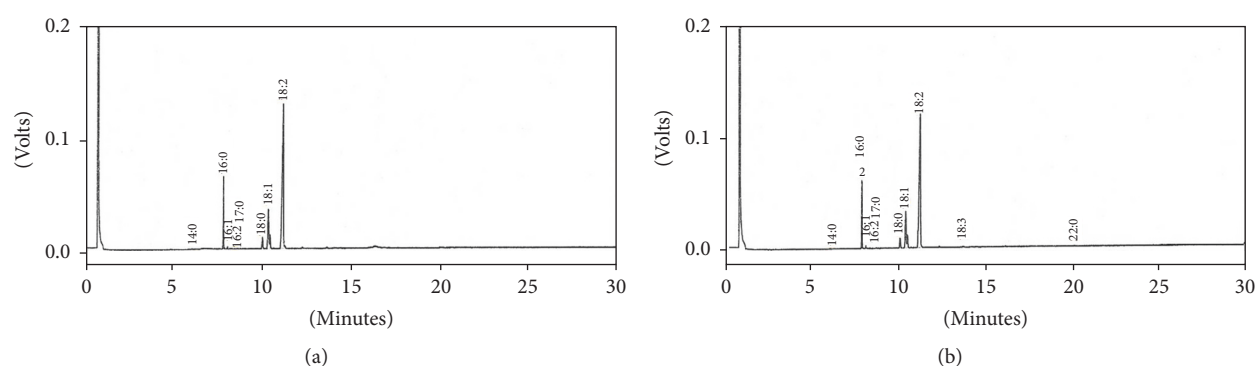


FIGURE 4: Chromatograms of FAMES of cactus pear seed oil extract. (a) Green cactus pear seed oil extract; (b) red cactus pear seed oil extract. In both oils extracts were identified: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1, *cis*-9), hexadecadienoic (C16:2, *cis*-9, 12), margaric (C17:0), stearic (C18:0), oleic (C18:1, *cis*-9), linoleic (C18:2, *cis*-9, 12), except linolenic (C18:3, *cis*-6, 9, 12), and behenic (C22:0) fatty acids that were identified only in red cactus pear seed.

antimicrobial activity. The membrane disruption could be one mechanism of action by inactivating microbial adhesion, enzymes, and proteins transport [15, 46]. RCPS and GCPS extracts inhibited most of the evaluated bacterial and fungi species (Table 1); however, antimicrobial activity was not detected for *Salmonella Typhi*, which is a gram-negative bacterium. In general, gram-negative bacteria have an effective outer membrane that restricts the penetration of amphipathic compounds and has a mechanism to extrude toxins across [50]. This may explain the apparent antimicrobial ineffectiveness of the oils against the permeability barrier in addition to the presence of multidrug resistance encoding plasmids [51].

3.4. Fatty Acid Profile. FAMES chromatograms and percentages are shown in Figure 4 and Table 2. Cactus pear seed oils contained saturated and unsaturated fatty acids, the linoleic fatty acid being the predominant (67.4% and 65.4% in GCPS and RCPS oils, resp.). Minimal amounts of myristic (C14:0), palmitoleic (C16:1), hexadecadienoic (C16:2), and margaric (C17:0) fatty acids in both oils were also identified. The fatty acids profiles of the two cactus pear varieties were similar; however the GCPS had a slightly higher content of the linoleic acid (C18:2) while the fatty acids linolenic (C18:3) and behenic (C22:0) were in minimal amounts only in the RCPS.

Different studies have established that factors as cultivar type, temperature, and harvest time have a strong influence in parameter as pH, Brix, vitamin C, sugars, and fat content [52, 53]. Oumato et al. [52] found differences in linoleic fatty acid (C18:2) content among cactus pear cultivars. In other study, the oleic acid (C18:1) content was significantly influenced by the cultivar and location interaction [53], providing unique characteristics to the oil.

In comparison with other plants oils, the linoleic acid (C18:2) content of the cactus pear fruit was similar to the levels reported for sunflower oil (62%) [54] and higher than wheat germ oil (55.05%) [55] and soybean oil (52.70%) [56]. The contents of other FAMES in cactus pear varieties such as palmitic (C16:0), oleic (C18:1), and stearic (C18:0) were similar to those reported for Castilla blackberry (*Rubus glaucus* Benth) with 11.24%, passion fruit (*Passiflora edulis*) with 15.47% [57], and grape (*Vitis vinifera*) with 3.5% [58].

Other researchers have reported similar fatty acids profile to our findings for different plant materials and have analyzed the antimicrobial effectiveness against different microorganisms. For instance, fatty acids found in *Allium cepa* were found to effectively inhibit *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, and *Klebsiella pneumoniae* [59]. Oil extracted from *Swietenia Macrophylla* king seed oil inhibited growth of *S. aureus*,

S. Typhimurium, and *P. aeruginosa* [60]. These studies demonstrate that seed oil can inhibit fungi and bacteria, but their efficacy would depend on their concentration levels and specific pathogen [15].

4. Conclusions

Oil yield from the green cactus pear was higher in comparison to the red cultivar and was also influenced by the solvent used. Hexane exhibited high extraction yield while oils extracted with ethanol had the better antioxidant activity. The results demonstrated that oil extracts from both varieties have a noticeable antimicrobial activity against gram-positive and gram-negative bacteria comparable to antimicrobial compounds such as ampicillin, streptomycin, and sulfamethoxazole/trimethoprim. This research provides further incentives to develop additives for the food, cosmetic, and pharmaceutical sectors seeking natural compounds with antimicrobial activity. Further studies are needed to determine the specific component responsible for the antimicrobial activity in cactus pear seeds oil and determine the optimum levels of oil extract and the antimicrobial effectiveness in the food matrix.

Additional Points

Practical Application. Our results suggest that the oils extracted from cactus pear seeds have the potential to be used as a natural antioxidant and antimicrobial agents by the food, cosmetic, and pharmaceutical sectors.

Conflicts of Interest

The authors have declared that no conflicts of interest exist.

Acknowledgments

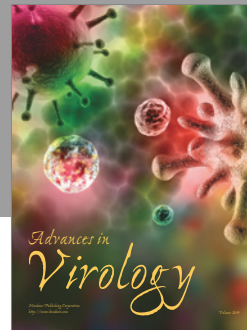
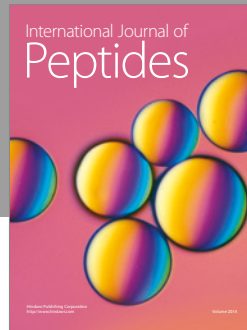
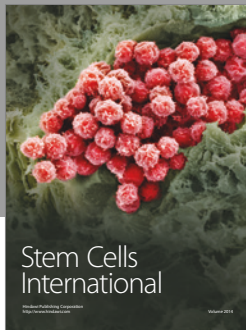
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